

Ce milieu coulé dans des pétris est inoculé à la pincette par un fragment du thalle T1. Pendant les dix premiers jours, le thalle se répand sur toute la surface offerte. Il s'agrippe aux fibres du papier. Dès le dixième jour, les sclérotés se forment. La première phase est terminée. Cette phase de croissance est certainement aérobie, l'air contenu dans les pétris étant suffisant. Dans la deuxième phase nous pouvons supposer un état d'anaérobiose partiel. La souche produit elle-même du CO_2 qui peut s'amasser dans le milieu fermé. Le champignon reste vivant, il est repiquable et continue à se transformer, mais il digère surtout toute la cellulose mise à sa disposition. Cette décomposition de la cellulose demande un à deux mois après lesquels le milieu devient transparent. Il nous reste à étudier le mécanisme de cette action. Au cours de la deuxième phase, le champignon libère-t-il dans le milieu les enzymes responsables, sans pour cela utiliser les produits de la dégradation, puisqu'à ce moment le maximum du développement est atteint?

Selon une autre hypothèse on pourrait croire à une transformation dirigée par le champignon qui se trouverait dans l'obligation de faire participer à son propre métabolisme les substances dégradées provenant de cette deuxième phase.

Par des expériences faites *in vitro*, du type de celles effectuées par REESE et col.¹, il sera sans doute possible de déterminer le genre de *cellulase*, dont nous postulons l'existence, active dans ce cas particulier.

M. A. ROULET

Institut et Jardin botanique de l'Université de Berne, le 22 août 1953.

Summary

A strain of Fungus was isolated which is able to live on media containing monosaccharides, polysaccharides, cellulose and lignin. We hope to study one of the mechanisms of cellulose degradation by cellulase.

¹ E. T. REESE et H. S. LEVINSON, *A comparative study of the breakdown of cellulose by microorganisms*, *Physiologia plantarum*, Vol. 5 (1952), p. 345.

Spreading Factor and Mucopolysaccharides in the Central Nervous System of Vertebrates

Preliminary observations concerning the so-called interstitial spaces and their contents in the central nervous system of vertebrates are now the subject of interest in a published series of brief reports¹.

Hyaluronidase enzyme action shows that the administration of this enzyme with injected liquids helps the spreading process within the so-called interstitial spaces of the central nervous system tissues (DURAN REYNALD's Spreading Factor) (Figure); whereas, all injected liquids without enzymes provoke immediate breaking-up or destruction of nerve centres.

The behaviour of nerve centres to the spreading factor was demonstrated in a series of experiments made on nervous tissue of many species of mammalia.

The spreading factor test on the nervous system of small vertebrates was replaced by the dissociation test of these enzymes on the nerve centres, that is, within a few hours after treating small fragments of nervous tissue with hyaluronidase enzyme the nervous tissue was easily dissociated².

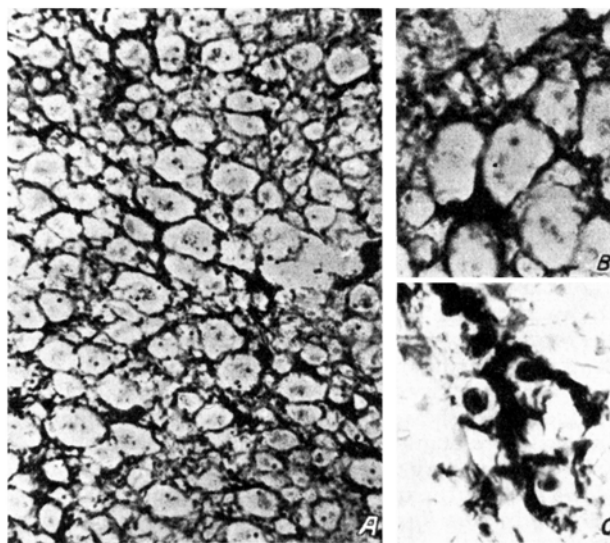
¹ A. BAIRATI and G. MATTIOLI, *Boll. Soc. it. Biol. sper.* 27, 127 (1951); *Atti Soc. it. Anat. Pavia* 1951, pubbl. in *Monit. Zool. it. Suppl.* 60, 101 (1952).

² A. BAIRATI, *Boll. Soc. it. Biol. sper.* 28, 1143 (1952).

The well defined behaviour of the connective tissues in respect to hyaluronidase enzymes suggested the possible analogy that mucopolysaccharides may be embedded in the so-called interstitial spaces of the central nervous tissues. We, therefore, arranged a series of histochemical experiments to attempt to show the existence and localization of this ground substance in the nervous centres in the different species of phyla. These experiments required the collaboration of many research workers attached to this Institute of Human Anatomy¹. The details of these experiments and observations were the subject of separate published reports².

Brief results and conclusions

(1) In all nervous systems of vertebrates so far tested, it was possible to demonstrate that the cohesive materials between nerve cells, fibres, glia and blood vessels are sharply influenced by hyaluronidase enzymes. Modified cohesiveness of the aforesaid parts after enzyme treatment is revealed by an easy diffusion of the injected liquids (Figure) and by the dissociation of the fresh tissues.



Bos taurus spinal cord. The specimen has been injected with India ink and hyaluronidase. A, cross section of the white substance (120 \times); B, details of A, high magnification (500 \times); C, same as B: India ink is spread between glial cells (600 \times).

(2) HOTCHKISS reaction and metachromasia tests are most useful to demonstrate these cohesive materials. It is advisable, before attempting these colour tests, to eliminate all probable causes of error which may be brought about by nucleic acids, glycogen, and the glycolipids formed by the cerebrosides during fixation. Once these elements are taken into consideration, positive value and reliability may be attributed to the HOTCHKISS reaction and the metachromasia tests for the presence of mucopolysaccharides.

¹ A. BAIRATI, A. BAIRATI jr., E. BARTOLI, G. BERTACCINI, G. MARSICO, F. MASSARI, M. PATERNO, and G. TRIPOLI, *Boll. Soc. it. Biol. sper.* 28, 1447 (1952).

² A. BAIRATI, *Atti Acc. Pugliese Sci.* 11, 195 (1952). – A. BAIRATI and G. TRIPOLI, *Atti Soc. it. Anat. Napoli* 1952, pubbl. in *Monit. Zool. it. Suppl.* 61, 105 (1952) (amphibia). – A. BAIRATI jr. and M. PATERNO, *ibidem* (fishes) pag. 107. – E. BARTOLI and G. BERTACCINI, *ibidem* (birds) pag. 109. – F. MASSARI and G. MARSICO, *ibidem* (mammalia) pag. 110.

(3) HOTCHKISS reaction and metachromasia have given clear positive slide images in the central nervous systems of fishes, amphibia and lower mammalia such as rodents and chiroptera¹. Doubtful and weak colorations were obtained with the central nervous system of birds and higher mammalia².

(4) Histochemical colouring methods may not give true images on structure and position of metachromatic and HOTCHKISS positive substances.

It is well known that these histochemical reactions involve precipitated material in a form which is probably far from what it is in real life.

Nevertheless, slide images obtained by infiltration of coloured materials (such as India ink), in the nervous tissue centres and successive glia coloration clearly help to affirm that hyaluronidase sensitive substances are lodged in the so-called interstitial spaces of the central nervous system.

(5) The data hereby reported agree with the old ideas expounded by such anatomist as DEITERS, VIRCHOW and GIERKE³ whereby the existence of an homogeneous substance between the neuroglia, nerve cells, fibres and axon terminations.

(6) Systematic comparison between histochemical reactions and glia make-up, show, up to now, that the intercellular matrix of white matter has a certain antagonism for histochemical reactions and fibrous glia development. Nevertheless, when the fibrous glia are highly developed the histochemical reactions are very weak.

As our researches were approaching completion, many other authors reported works with data agreeing in many respects with our experimental results which will soon be subject of a separate report.

FREEDMANN⁴ has published a brief report whereby the application of his experimental method demonstrates the existence of Hyaluronidase/Hyaluronic acid systems in the intercellular matrix of the gray matter of the central nervous system.

HESS⁵ has shown, with his histochemical methods, the existence of mucopolysaccharides in the intercellular matrix of the gray matter of the central nervous system of the different mammalia. In all probability the mucopolysaccharides form the ground substance of the central nervous system. The experimental data presented by HESS agree fully with the results obtained from our researches.

A. BAIRATI

*Institute of Human Anatomy, University of Bari, Italy,
July 16, 1953.*

Résumé

Réactions histochimiques et enzymatiques, l'action positive du facteur diffuseur de DURAN-REYNALDS montrent que dans les espaces intercellulaires du système nerveux central des vertébrés existent des substances mucopolysaccharides. La glie (VIRCHOW) est constituée par une substance amorphe mucopolysaccharide et par la texture glie-vasculaire.

¹ A. BAIRATI jr. and M. PATERNO, Atti Soc. it. Anat. Napoli 1952, pubbl. in Monit. Zool. it. Suppl. 61, 107 (1952) (fishes). – E. BARTOLI and G. BERTACCINI, ibidem (birds) pag. 109. – H. GIERKE, Arch. mikr. Anat. 25, 95 (1885); 26, 110 (1886).

² F. MASSARI and G. MARSICO, Atti Soc. it. Anat. Napoli 1952, pubbl. in Monit. Zool. it. Suppl. 61, 110 (1952) (mammalia). – H. GIERKE, Arch. mikr. Anat. 25, 95 (1885); 26, 110 (1886).

³ H. GIERKE, Arch. mikr. Anat. 25, 95 (1885); 26, 110 (1886).

⁴ B. FREEDMANN, Anat. Rec. 115, 265 (1953).

⁵ A. HESS, J. Comp. Neurol. 98, 69 (1953).

The Significance of Temperature and the Daily Light – Dark Period in the Formation of Resting Buds

As has already been shown, increased storage temperatures are able to produce in resting winter buds a further deepening of dormancy¹. Furthermore, those buds which had recently emerged from dormancy could be rendered dormant again by raising the temperature sufficiently², this secondary resting period exhibiting all the typical characteristics of the first "natural" one. These observations, coupled with the fact that in the case of most plants the resting period begins in the summer, have convinced the author that the "natural" resting period is mainly due to the influence of high summer temperatures³.

In the present communication, an attempt has been made to investigate the influence of temperature and photoperiodism upon the formation of resting buds. It has become clear that here, too, temperature is the main factor determining the occurrence or non-occurrence of such dormancy. Within certain limits, however, the effect of this factor is suppressed by the duration of the daily light period.

The experimental material comprised plants of *Hydrocharis morsus ranae* L. which had been allowed to develop from winter buds, the dormancy of the latter having been broken by storing them at 5°C. The plants were maintained in tap water at temperatures of 10, 15, 20, and 25°C, respectively, both in the dark and under artificial daylight. The daily periods of illumination lasted, respectively, 3, 6, 9, 12, 15, 18, and 21 h, as well as continuous illumination.

The results clearly indicate that the environmental temperature is of decisive importance in inducing the formation of dormant buds. At 10°C, and independent of the length of the daily light-period, no resting buds occur, the plants continuing their growth by the production and development of non-resting, stolon-forming buds. On the other hand, resting buds tend to occur at 15, 20, and 25°C, though only when the daily light-period is not too long.

From a photoperiodic standpoint, the formation of resting buds at the above temperatures must be considered a short-day character, i.e., it occurs when the duration of the daily light-period is neither too long nor too short. Resting buds are not produced under conditions of continuous illumination. Here a decisive factor in the initiation of resting-bud formation is the duration of the daily dark-period, the length of which must not lie below a definite minimum value.

Resting buds are also formed by plants maintained entirely in the dark, on a saccharose solution, at 18–22°C room temperature. From this it may be concluded that the significance of the daily light-period lies only in its effect on the synthesis of carbohydrates which are necessary not only for the formation of resting buds but also for storage in the latter or in the adjacent tissues.

If the duration of the daily light-period exceeds the length characteristic for each temperature, light inhibits the formation of resting buds. Where the daily light-period is too long, i.e., where the corresponding dark-period is too short, plants maintained at temperatures of 15°C and higher continue growth by the formation of non-resting stolon-forming buds.

¹ A. VEGIS, Symbolae Bot. Upsalienses 10, 2 (1948).

² A. VEGIS, Physiol. Plant. 2, 117 (1949).

³ A. VEGIS, Svensk Bot. Tidskr. 43, 671 (1949).